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Morphometry of Medial Gaps of Human Brain Artery Branches

Peter B. Canham, PhD; Helen M. Finlay, BSc

Background and Purpose—The bifurcation regions of the major human cerebral arteries are vulnerable to the formation of saccular aneurysms. A consistent feature of these bifurcations is a discontinuity of the tunica media at the apex of the flow divider. The objective was to measure the 3-dimensional geometry of these medial gaps or “medial defects.”

Methods—Nineteen bifurcations and 2 junctions of human cerebral arteries branches (from 4 male and 2 female subjects) were formalin-fixed at physiological pressure and processed for longitudinal serial sectioning. The apex and adjacent regions were examined and measurements were made from high-magnification photomicrographs, or projection microscope images, of the gap dimensions at multiple levels through the bifurcation.

Results—Plots were made of the width of the media as a function of distance from the apex. The media at each edge of the medial gap widened over a short distance, reaching the full width of the media of the contiguous daughter vessel. Medial gap dimensions were compared with the planar angle of the bifurcation, and a strong negative correlation was found, ie, the acute angled branches have the more prominent medial gaps.

Conclusions—A discontinuity of the media at the apex was seen in all the bifurcations examined and was also found in the junction regions of brain arteries. We determined that the gap width is continuous with well-defined dimensions throughout its length and average length-to-width ratio of 6.9. The gaps were generally centered on the prominence of the apical ridge. (Stroke. 2004;35:1153-1157.)

Key Words: cerebral aneurysm ■ connective tissue disorders ■ cerebral arteries ■ histomorphometry

The branching regions of major cerebral arteries are disproportionately involved in vascular pathology compared with the remainder of the vascular network. They are, for example, vulnerable to the development of saccular aneurysms, balloon-like expansions that may enlarge and eventually leak or fail catastrophically. The goal of identifying what factors might signal the higher-risk aneurysms, factors such as vascular structure or geometry, blood flow mechanics, or genetics, has motivated a substantial body of research.1–6

Human brain arteries are muscular arteries, comprising 3 main layers: the outer tunica adventitia with a strong presence of type I collagen, the tunica media dominated by smooth muscle with an extracellular matrix of types I and III collagen fibers, and an inner layer, the tunica intima, that has a layer of endothelial cells adjacent to the lumen, separated from the media by a distinct internal elastic lamina (IEL).6,7 With aging or as a result of pathological factors, the intima commonly acquires a thickened subendothelium. Unlike most muscular arteries, brain arteries do not have an external elastic lamina. Various theories have been put forward with respect to the cause of saccular aneurysms, including the possibility of a disruption of the IEL, and the potential effect of hemodynamic pressure and flow.8,9 A well known distinctive microscopic feature of brain arteries is the discontinuity of the media at the apex of cerebral artery bifurcations that has been referred to as the medial defect, medial gap, or raphe.7,9–12 A medial gap by itself is unlikely to be the defining structure leading to aneurysm formation, because of the gap’s frequent presence in human systemic arteries and in animal arterial bifurcations, where saccular aneurysms are relatively rare.7 However, because the medial gap is almost always present at the bifurcation, any investigation into the cause of saccular aneurysms must take into account the structural implications of the morphology of the apex.

The purpose was to investigate the geometry of the cerebral artery bifurcation, addressing particularly the medial gap. By graphing the width of the media on both sides of the flow divider as a function of distance from the apex, we learned: (1) of the consistent geometry of the tapering media on either side of the apex; and (2) that the width of the medial gaps increases significantly with a narrower branching angle. We included examples of “arterial fenestrations,” which are fusion anomalies in the development of some brain arteries in which a trunk artery has a short segment of side-by-side separate arteries that re-converge. Fenestrations thus represent examples of flow dividers and convergences in close proximity, and they, too, have medial gaps.7

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Methods

The study was divided into 2 main parts. The first was to examine the shape and dimensions of the tunica media as it tapers to the gap and to look at the medial gap itself. The second was to assess in detail how the shape of the media changes as the gap extends through the apex from one side to the other and to explore a relation between the angle of the bifurcations and the medial gap dimensions. Measurements were made also of the tunica media dimensions at the lateral angles of 8 bifurcations, regions reported to be additional sites of media thinning.

Brain arteries from the circle of Willis were taken from 6 autopsies, 4 males and 2 females, age 55 to 73 years. For the primary study, 17 separate branch regions were obtained from 5 of these autopsies. These included 1 posterior cerebral artery, first bifurcation; 4 anterior cerebral arteries, 3 from the first main bifurcation in the distal branch and 1 more distal; and 12 middle cerebral arteries, 7 from the first bifurcation, 2 from the second and 3 from more distal branches. From the sixth autopsy, it was found that a region of the basilar artery, just distal to the vertebral junction, was segmented into a fenestration for a distance of ~1.8 mm. We used 4 separate regions, including the convergence of the vertebral arteries, the bifurcation at the leading edge of the fenestration, its convergence back to the basilar artery, and a further bifurcation to the anterior inferior cerebellar artery. For 2 of the autopsies, a method of whole-brain perfusion, with 10% buffered formalin at 100 mm Hg, was used to pressure-fix the arteries;13,14 from the other 4 autopsies, isolated lengths of artery including the bifurcations were cannulated and pressure-fixed. The pressure fixation is critically important in that it maintains the artery and branch in its physiological geometry. Segments of artery including branches were embedded in paraffin and sectioned longitudinally completely through the apex at ~7-μm thickness. Sections were stained with Gomori trichrome with aldehyde–fuchsin, revealing collagen as light blue/green, muscle cells as dark red, and elastin as dark purple, providing good contrast for half-tone micrographs. We have not taken into account deformation caused by paraffin embedding. Shrinkage has been measured for similarly prepared brain arteries at 8.7% radially and 14.5% longitudinally. Allowing that shrinkage is similar for all the bifurcations, similarly prepared brain arteries at 8.7% radially and 14.5% longitudinally, similar calibrations were performed by means of a stage micrometer. Seven bifurcations from 2 autopsies were used for this part of the study. The series of sections of each bifurcation was examined so that a mid-plane section could be selected from the region in which the medial gap was at its full width. From 1 of the bifurcations, we analyzed 2 sections separated by ~650 μm, and from another we examined 4 adjacent sections to give an indication of the variance among sections. Continuous measurements were made of the width of the media along the 2 daughter vessel walls (~40 data in each section), and these were plotted against the perimeter distance from the apex. These plots do not include any information about the branch angle between the 2 daughter vessels. A schematic presentation of the sectioning planes, the 3-dimensional view of the medial gap in the branch, and the location of measurements are shown in Figure 1.

Figure 1. a. Schematic lateral view showing sectioning planes and the lateral principal curvature of the apex. b. View to illustrate extent of medial gap. c. Mid-plane diagram showing areas of measurement. d. Diagram of branching vessel with high bifurcation angle, a.

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The length or extent of the medial gap was calculated from section thickness and a count of the number of sections along the apex of the flow divider, from the first section in which the 2 parts of the media became separate, to the section on the opposite side of the apex in which they became reconnected. At either end of these sets, there were sections in which the media was present but was narrowed. By measuring the width of the gap at several positions through the apex, we were able to get an assessment of its shape.

A second part of the study was to investigate how the width of the medial gap and the shape of the media tapering changed as sections were analyzed through the apex (Figure 1a). Micrographs were taken of the bifurcation apex from between 3 and 30 sections through each bifurcation, and a stage micrometer was also included so that an accurate scale factor could be applied. Twelve bifurcations and 2 junctions from 6 autopsies were examined, and dimensional measurements made directly from the micrographs (magnification 300× to 500×):15 The distance from the point of the apex was measured at increments of 5 mm along the edge of the IEL. At each of these points, the width of the media was measured to 0.5 mm on the micrographs (ie, 10- to 15-μm accuracy). The data were plotted as in Figure 1, but they were also plotted in such a way that the origin of each analyzed histological section was offset on the y-axis by an amount equivalent to the calculated distance of that section from the previous section, calculated by the number of sections multiplied by 7 μm. We note that the gap width is a true dimension in all sections, whereas the layer thickness of the media is a true dimension on or near the mid-plane sections; however, it appears larger for sections cut significantly away from the mid-plane because the cutting plane becomes more tangential.

Measurements were made of the angles of the vessels, both to indicate the total divergence and to reveal any differences in the angle of divergence of the 2 daughter vessels from the main trunk. These angles were obtained by using tracings of the complete bifurcations. From each of the parent and 2 daughter vessels, a distance from the apex equal to the diameter of the vessel was estimated and the luminal axis within that length of the vessel was drawn.

Results

Micrographs of flow dividers with narrow and wide branching angles are shown in Figure 2, with the direction of blood flow being upward in all cases. Media muscle is seen in dark contrast, with the IEL showing as a black continuous band. The first 5 micrographs (Figure 2a through 2e) show apex regions of 3 narrow bifurcations, and 2 with wider angles. Also included are a vertebral artery convergence (Figure 2f), the leading edge of a basilar artery fenestration (Figure 2g), and the distal convergence of the fenestration (Figure 2h). In both convergences, the medial gap is similar in shape and dimensions, and a prominent thickened intimal layer can be clearly seen.
The graphs of Figure 3 show plots of the width of the media, starting from the apex as a center point, for 7 bifurcations of brain arteries from 2 different autopsies. Included in the upper graph is an example of an off-center gap, MCA1, (as in Figure 2a and 2b). There is a consistent and abrupt termination, within 100 to 200 μm, of the medial smooth muscle fibers on both sides of the apex. When 4 adjacent sections of an ACA bifurcation were analyzed, we were able to calculate the precision of the method. The variability includes the slight biological variation within the 21 μm that separated the sections and the errors associated with the histological methods and analysis. Separate calculations of the left and right media widths gave standard deviations of 5.1 μm and 6.5 μm, respectively. Over this same series of 4 sections, the width of the gap was 0.167 mm ± 0.034 mm.

For the second part of the study, we assessed the 3-dimensional aspect of the medial gap, for which we used the sets of serial sections with detailed measurements at discrete intervals through the depth of the bifurcation region (Figure 1a). Graphs in Figure 4 show the results for an MCA (5 sections) and a leading edge of a basilar artery fenestration (4 sections). The depth location of each section is presented to scale along the y-axis. The MCA bifurcation in Figure 4a is an example with daughter vessels having different media widths, with one vessel being approximately twice as thick as the other. A set of 4 plots (Figure 4b) is taken from the apex of the proximal part of a fenestration in a basilar artery, in which both “branches” are approximately equal in diameter, and the 2 sides of the apex can be seen to be similar.

Artery junctions were also measured, with the convergence of vertebral arteries forming the basilar artery and the junction at the distal end of the fenestration of the same basilar artery (Figure 2f and 2h). Both had medial gaps similar to those of bifurcations, although the shape of the apex of the convergence was built-up and round, with a considerable amount of thickening of the subendothelium that is not seen in the flow divider of bifurcations (ie, a structural factor related to flow direction).

Measurements were made of the width and the length of the medial gap from 15 bifurcations. The maximum width varied from 0.038 to 0.225 mm, with an average of 0.12 mm, and the length varied from 0.42 to 1.40 mm, with an average of 0.85 mm. The calculation of length was made by counting the number of sections. Because of the obliqueness of the cutting plane at either end of each gap, these values will be an underestimate. The gap extended above and below the mid-plane by a mean distance of ≈60% of the trunk vessel radius. If the assumption is made that the curvature of the apex is approximately circular, then it is calculated that the length is underestimated by 7%. Thus, the medial gap has a narrow oval-like shape following the curvature of the flow divider.

Lateral angle medial gaps were also investigated. From 8 of the bifurcations, we measured the dimensions of the media of the 2 lateral angles from the sides of the main trunk where
it meets the daughter vessels (Figure 1c). In 11 of 16 lateral angles, there was thinning of 20–50%, and in 2 there was thinning of 50%. We did not find a complete gap in any of these samples. With further exploration of different bifurcations, however, we did find complete gaps in 2 lateral angles in which the daughter vessel had a large angle from the trunk vessel axis. One of these was the bifurcation to the anterior inferior cerebellar artery just distal to the basilar fenestration. This branch was much smaller in diameter than the basilar artery and was angled backward from the parent vessel (100° as illustrated in Figure 1d). Both the apex and lateral angles had a similar gap width, but the lateral angle had the subendothelial buildup typical of vessel junctions.

**Discussion**

The medial gap, characterized by the relatively abrupt termination of muscle cells of the media at the apex or flow divider of branching arteries was found in all bifurcations in the study. By fixing the specimens at arterial distending pressure, we had maintained the in vivo dimensions of the individual layers (avoiding the characteristic circumferential shortening and corrugated folding of the arterial wall in nonpressure-fixed vessels). Our measurements and graphical data have corroborated the extensive microscopical study of brain artery branches processed without pressure fixation. The gap was present in small and large vessel branches (the smallest in the study being a branch with diameter 0.5 mm) and was generally centered on the prominence of the apex in the longitudinal planar sections (centered and off-center examples shown in Figure 2). We explored the extent of the gap “above” and “below” the central sectioning plane and found that the gap has the 3-dimensional form of a tapered channel in the media, replaced by adventitial collagen tracking the smooth curvature of the arterial apex.

The geometry of the muscle cell gap is precisely repeated from section to section, illustrated in Figure 4a for 5 representative sections spanning 406 μm (gap dimensions from 16 to 24 μm, with the consistently thinner and more tapered media for the left branch [-ve axis]). The thickness of the media of the downstream vessels varies 20 to 30 μm, deviation encompasses structural differences within a blood vessel, measurement error is associated with the method, and morphometric artifacts are made apparent when cutting serial sections. The leading edge of a basilar artery fenestration shows similar geometric consistency for a narrower and more abrupt gap, 6 to 10 μm in width (Figure 4b).

The dimensional analysis of the study provided the opportunity to investigate correlations with other cerebral vascular parameters such as branch angles. Measured branch angles varied widely, ranging from 37° to 133°, with a mean planar branch angle of 74.4°. The relationships that revealed significant correlations were both the width and the overall length of the medial gaps when graphed against the planar angle of the bifurcations (Figure 5). The strong correlation between width of medial gap and branch angle predicts a reduced incidence of gap for very wide angled branches, as observed for lateral angles.
The lack of smooth muscle cells has not been explained and may be related to the unique geometry and cellular response to the local wall stresses. The presence of the defect might be a consequence of the circumferential arrangement of the medial tissue, the alignment of which would make it difficult to support a continuous layer through the apex of the division, particularly for sharp angled branches. Smooth muscle cells in the media of an unbranching segment of artery are highly aligned circumferentially. Thus, at the apex of a branch site, there are 2 separated parallel groups of muscle fibers belonging to the media layers of the 2 daughter vessels. Between these 2 parallel groups of fibers is the medial gap, in which the parallel tendon-like collagen fibers of the apical ridge and adventitia run. The upstream larger trunk vessel has media muscle fibers that can merge effectively with those of both daughter vessels at the lateral angles and elsewhere, but not near the apical region. This is illustrated by Walsmley’s findings of layered multidirectional smooth muscle fibers in the trunk vessel media, near the ends of the medial gap. If the smooth muscle cell network were to align in the direction of the dominant cyclic strain as they do in unbranching segments of muscular arteries, then no single pattern of alignment could meet the requirements in the elastically stiff apical region of a branch.

Muscle cell networks are responsive to their environment and are participants in vascular remodeling. The concept of remodeling over time has support from Stehbens’ research showing the increasing incidence of medial gaps with age. Although medial gaps have a wide distribution in animals and humans, they do figure prominently in the human cerebral circulation where aneurysms develop, and they must continue to be taken into account when considering the vascular remodeling that leads to aneurysm development.

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